

FBS08- Organic DNA Extraction

Table of Contents

1. Scope
2. Background
3. Safety
4. Materials Required
5. Standards and Controls
6. Calibration
7. Procedures
8. Sampling
9. Calculations
10. Uncertainty of Measurement
11. Limitations
12. Documentation
13. References

1. Scope

- 1.1. This procedure describes the isolation of deoxyribonucleic acid (DNA) from biological specimens recovered from evidentiary items for nuclear DNA typing.

2. Background

- 2.1. Biological samples contain a number of substances in addition to DNA. DNA molecules must be separated from other cellular material before they can be examined. Cellular proteins that package and protect DNA in the environment of the cell can inhibit the ability to analyze the DNA. Therefore, DNA extraction methods have been developed to separate proteins and other cellular materials from the DNA molecules.

3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.
- 3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

- 4.1. Digest Buffer (FBR35)
- 4.2. Proteinase K (FBR36)
- 4.3. Phenol/Chloroform/Isoamyl Alcohol (PCI)
 - 4.3.1. Note: This reagent and its waste must be handled in a fume hood.
- 4.4. TE Buffer (FBR14)
 - 4.4.1. NOTE: Never use solutions directly from the stock bottles. Use Reagent SOPs for preparation and labeling instructions.
- 4.5. Optional- Phase Lock Gel Tubes

5. Standards and Controls

- 5.1. At least one reagent blank (i.e., extraction control) must be prepared and processed in parallel with each set of evidentiary specimens for DNA typing purposes. The reagent blank(s) is comprised of all the reagents used in the analytical process and carried through the same extraction, quantitation, amplification and electrophoretic typing procedures as the evidence samples. If more than one extraction method is used then at least one reagent blank must be processed for each type of procedure. The reagent blank(s) implemented as part of the organic DNA extraction procedure performed on the evidence is named the RB. The RB should always be the last sample processed in a set.
- 5.2. In order to maintain a separation in time and space between questioned and known samples:
 - 5.2.1. At no time should questioned and known samples be simultaneously incubating in the same heat block.
 - 5.2.2. At no time should questioned and known samples be simultaneously extracted in the organic fume hood.

6. Calibration

- 6.1. Not applicable

7. Procedures

- 7.1. To each sample tube, pipette 400 μ L Digest Buffer and 12 μ L of Proteinase K (10 mg/mL) solution. Vortex and quick-spin in a microcentrifuge. Incubate at 56°C for a minimum of 2 hours to overnight.
- 7.2. **OPTIONAL: Additional Digest Buffer and Proteinase K, in the proper concentrations, may be added to the sample, if needed.**

- 7.3. Note: The Digest Buffer and Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.
- 7.4. After digestion, vortex and spin-down the sample tubes. Transfer the substrate to a filterless basket in a 2.0mL microcentrifuge tube and spin for 3 – 5 minutes at maximum speed. Save the substrate in a new sterile tube labeled with the case number, sample number, and “substrate remains.”
- 7.5. OPTIONAL: Obtain an appropriate number of phase lock gel tubes and label appropriately. Microcentrifuge at maximum speed for 20 to 30 seconds.
- 7.6. OPTIONAL: Add entire sample to phase lock gel tube.
- 7.7. In a fume hood add 500 µl of PCI solution. Thoroughly mix to form a transiently homogenous suspension.
- 7.8. Microcentrifuge the samples at room temperature for 5 minutes at maximum speed to separate the two phases. Proceed to Step 7.10 if the upper aqueous phase is clear.
- 7.9. Note: If the aqueous phase is not clear (e.g., cloudy, dark in color or colored from dyes), due to incomplete phase separation, then transfer the upper aqueous phase to a new sterile 1.5 ml microcentrifuge tube or phase lock gel tube. Repeat steps 7.7 – 7.8 an additional 2 to 3 times, until the interface is clean and the aqueous phase is clear. For these additional extractions, the lower Phenol/Chloroform/Isoamyl Alcohol layer may be removed and discarded, eliminating the need for a new microcentrifuge tube.
- 7.10. Assemble the Microcon and label the specimen reservoir.
- 7.11. Add 100 µL TE Buffer to upper reservoir to pre-wet the membrane.
- 7.12. Transfer the entire aqueous phase to the upper reservoir containing TE Buffer. Centrifuge at 500 x g for at least 10 minutes. Remove the upper specimen reservoir from the tube, discarded the effluent in the lower reservoir, and re-insert the upper reservoir into the Microcon tube. Alternatively, the upper reservoir may be placed into a new appropriately labeled Microcon tube and the original tube containing the effluent discarded.

- 7.13. Add 200 μL of TE Buffer to the upper reservoir.
- 7.14. Centrifuge at 500 x g for at least 10 minutes. If liquid remains, additional spin(s) may be performed.
- 7.15. Label a new set of Microcon tubes with a case number and sample number.
- 7.16. Add an appropriate volume (approximately 25 μL and 100 μL) of TE Buffer to the specimen reservoir.
- 7.17. Remove the specimen reservoir from the initial reservoir tube. Invert the specimen reservoir and place it into a new Microcon tube. Centrifuge at 500 x g for 3 to 5 minutes to elute the concentrated DNA. (Be certain that the caps of the tubes are all facing inward in the microcentrifuge to avoid possible snapping of the cap).
- 7.18. Discard filter and close final Microcon tube. Final extract volume should be approximately 25-100 μL . The contents of the final Microcon tube may be transferred to an appropriately labeled sterile extract tube.
- 7.19. Store the samples frozen.
- 7.20. If needed, a sample may be re-concentrated following steps 7.10 - 7.18.

8. Sampling

- 8.1. Not applicable

9. Calculations

- 9.1. Not applicable

10. Uncertainty of Measurement

- 10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL*

Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6).

11. Limitations

- 11.1. The quantity and quality of the DNA present within any biological material ultimately determines if a nuclear DNA isolation is successful.
- 11.2. All nuclear DNA isolation steps in which Phenol/Chloroform/Isoamyl Alcohol is used must be performed in a fume hood.
- 11.3. To ensure a homogeneous solution, the Phenol/Chloroform/Isoamyl Alcohol reagent should be removed from the refrigerator and allowed to equilibrate to room temperature before beginning extractions.
- 11.4. Caution should be taken to prevent microcon filters from exceeding their specified limitations. Excessive g-force may result in leakage or damage to the centrifugal device.

12. Documentation

- 12.1. Organic Extraction Sample Sheet

13. References

- 13.1. Comey, C.T., Koons, B.W., Presley, K.W., Smerick, J.B., Sobieralski, C.A., Stanley, D.M., and Baechtel, F.S. DNA extraction strategies for amplified fragment length polymorphism analysis. *Journal of Forensic Sciences* (1994) 39: 1254-1269.
 - 13.1.1.
- 13.2. Millipore Corporation. Microcon[®] Centrifugal Filter Devices User Guide. Millipore Corporation, Billerica, MA, 2000.
- 13.3. Forensic Science Laboratory Quality Assurance Manual (Current Version)
- 13.4. FSL Departmental Operations Manuals (Current Versions)
- 13.5. FSL Laboratory Operations Manuals (Current Versions)
- 13.6. FBR35 - Digest Buffer (Current Version)

13.7. FBR36 - 10 mg/ml Proteinase K in 10mM Tris pH 8.0 (Current Version)

13.8. FBR14 - TE Buffer (Current Version)